

EVALUATION OF RESPONSE TO TYROSINE KINASE INHIBITORS IN RENAL CELL CARCINOMA PATIENTS BASED ON EXPRESSION OF miR-99b, -144, -210, -222, -302a AND -377 IN TUMOR TISSUE

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Background: Renal cell carcinoma (RCC) is one of the most common solid tumors in adults highly resistant to conventional therapies. The expression profile of a number of miRNAs correlates with RCC response to chemotherapeutic agents. **Aim:** To identify the association of tumor miRNAs expression with neoadjuvant treatment response in patients with RCC. **Materials and Methods:** We analyzed the expression levels of tumor miR-99b, -144, -155, -210, -222, -302a, -377 in 93 RCC patients who received pazopanib or sunitinib in neoadjuvant regimen using RT-PCR. RNU48 was used as a reference miRNA. **Results:** The levels of expression of miR-99b and -377 are associated with the RCC response to pazopanib, and microRNA-210 and -377 to sunitinib. The characteristic expression profile of miR-99b, -144, -222, -377, and miR-302a determined in 90% of cases was delineated in pazopanib responders as opposed to nonresponders. Similarly, the characteristic expression profile of miR-210, -222, -302a and -377 was suggested for sunitinib responders. **Conclusions:** Levels of miR-99b, -210 and -377 expression in RCC tumor tissue might be used as a basis for future predictive panel intended for the assessment of the sensitivity to the regimens of neoadjuvant RCC treatment. **Key Words:** miRNA, renal cell carcinoma, targeted therapy.

DOI: 10.32471/exp-oncology.2312-8852.vol-43-no-2.16383

Renal cell carcinoma (RCC) is characterized by a variable and unpredictable clinical course due to its genetic heterogeneity and morphological diversity [1]. Despite the significant improvement in the detection of kidney cancer in early stages, about 40% of patients came with the advanced disease with metastases; 20–40% of cases are diagnosed with the disease progression with recurrence even after radical surgical treatment [2]. Today, the effectiveness of RCC treatment is low (at stages I, II, III and IV of the disease 5-year survival rates are 81%, 63.5%, 43% and 9.3%, respectively). Low survival rates of the patients with advanced kidney cancer may be due to the fact that the treatment standard for such patients is nephrectomy with or without systemic therapy. However, nephrectomy is associated with an increased risk of renal failure, increased cardiovascular disease risk and the risk of death from the consequences after surgery [3].

No less important is the treatment of patients with metastatic renal cell carcinoma (mRCC). In the era before targeted therapy, cytoreductive nephrectomy was preferred for mRCC treatment [4]. In addition, cytoreductive nephrectomy, as a primary procedure, could not be the standard of care for patients with poor general condition, poor or moderate prognosis according to International Metastatic Renal Cell Carcinoma Database Consortium/Memorial Sloan Kettering Cancer Center [5]. However, it can be performed in

patients with single metastases, for palliative purpose or in patients with a good response or stabilization of the process after primary systemic therapy [6].

Despite the fact that the systemic therapy has certain advantages in patients with mRCC, its role in the preoperative treatment of locally advanced kidney cancer with the ultimate goal of reducing tumor size and improving surgical resectability remains unclear. There are numerous studies in the literature, but there is a large heterogeneity of analyzed groups (tumors at different stages) [7].

The issues of choosing the optimal drug and the effectiveness of systemic therapy in the neoadjuvant regimen remain unexplored, as well as there are no reliable predictive markers that can be used for drug selection. In this aspect, the search for predictive miRNAs is one of the major directions of RCC research [8].

MiRNAs are involved in the regulation of all cellular processes. The impaired miRNA expression affects the characteristics of the tumor, i.e., cell growth and survival, angiogenesis, apoptosis [9]. Recent studies have conclusively shown that changes in the epigenetic regulation of genes in the metabolism of xenobiotics and target proteins play a key role in the development of drug resistance and determine the effectiveness of breast cancer therapy [10]. The effectiveness of therapy with targeted drugs depends on the expression of their target proteins. In recent years, clinical observations have linked miRNA levels with sensitivity to sunitinib and pazopanib, but these studies are isolated and not systematic [11].

The introduction of a new diagnostic algorithm and reasonable indications for targeted therapy in

Submitted: May 07, 2020.

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Abbreviations used: mRCC – metastatic renal cell carcinoma;
RCC – renal cell carcinoma.

RCC patients will significantly increase the treatment effectiveness, prolong duration, reduce disability of this contingent of patients [12]. Thus, the aim of our research was to identify the association of tumor miRNAs expression with neoadjuvant treatment response in RCC patients.

MATERIALS AND METHODS

We have performed retrospective analysis of postoperative material (tumor tissue) of 93 patients who underwent neoadjuvant systemic targeted therapy and surgical treatment for clear cell RCC in 2015–2020. All patients provided an informed consent on the use of clinical data for scientific purposes. The study was approved by the Institutional Ethics Committee of the National Institute of Cancer, Kyiv, Ukraine (record No. 163 of June 23, 2020). All samples were encoded and depersonalized. The clinical and pathological characteristics of the patients are presented in Table 1.

Our study included patients with localized RCC who received targeted therapy in neoadjuvant regimen [13]. All patients had a high risk of nephrectomy (RENAL score — 10–12) [14]. In this regard, tumor biopsy was performed in order to confirm the diagnosis morphologically and to assess the possibility of organ-sparing treatment. The clear cell RCC was diagnosed and neoadjuvant therapy with drugs of the first line of therapy sunitinib or pazopanib was performed [15].

To evaluate the efficiency of neoadjuvant therapy, patients were divided in two groups: the first included 79 (84.9%) patients who received pazopanib 800 mg

daily for 2 months; the second group were 14 (15.1%) patients, who during 10 weeks received sunitinib 50 mg daily with a two-week break between cycles. Regression of kidney tumor was determined by RECIST 1.1 [16] based on the results of spiral computed tomography or magnetic resonance imaging before and after neoadjuvant therapy.

The expression of miR-99b, -144, -155, -210, -222, -302a, -377 in tumor tissue was analyzed by reverse transcription polymerase chain reaction (RT-PCR) in real time. Total RNA was extracted from formalin fixed paraffin embedded tumor samples using commercial “RNeasy FFPE Kit” (Qiagen, Germany) from tumor samples. The amount of isolated RNA was determined on a “NanoDrop 2000c” spectrophotometer (ThermoScientific, USA). The purity of isolated RNA was controlled using the correlation values of optical absorption at the wavelength of 260 and 280 nm. RNA was dissolved in Tris-EDTA buffer and stored at –20 °C. Quantitative PCR (qPCR) was performed on a QuantStudio 5 Dx Real-Time PCR System (ThermoScientific, USA) with a commercial Maxima™ SYBR Green/ROX Master Mix (2 X) PCR kit (ThermoScientific, USA) according to the manufacturer’s protocol. To determine miRNA expression, we used a single stem-loop primer for synthesis of cDNA and universal reverse primer 5′-GTGCAGGGTCCGAGGT-3′, according to the methods of stem-loop miRNA RT-qPCR [17]. Sequences of primers were obtained by using the resource <http://genomics.dote.hu:8080/mirnadesigntool/> and synthesized by Metabion, Germany (Table 2).

The expression of miRNAs was determined using the 2^{–ΔCT} method relative to RNU48 miRNA used as a reference gene (endogenous control) [18]. Primer sequences were taken from <https://www.ncbi.nlm.nih.gov> and synthesized by Metabion, Germany: RT-primer: 5′-CTCTGACC-3′, forward 5′-AGTGATGATGACCCAGGTAAGT-3′, reverse 5′-CTGCGGTGATGGCATCAG-3′. ΔCT (delta threshold cycle) values were calculated automatically by QuantStudio 5 Dx Real-Time PCR System (ThermoScientific, USA) by following formula:

ΔCT = CT (a target gene) – CT (a reference gene).

All values of miRNA expression are presented as 2^{–ΔCT}.

Statistical processing of the results was performed using GraphPad Prism 4.0 software. Descriptive statistics included the calculation of the mean with standard deviation. Comparison of quantitative indicators in

Table 1. Clinical and pathological characteristics of RCC patients

		Number of patients	
		n	%
Age (range; mean ± SE), years		26–79 (56.7 ± 9.7)	
Gender	Male	64	68.82
	Female	29	31.18
	Tumor size (range; mean ± SE), mm	38–170 (74.3 ± 23.3)	
Pathological stage			
pT1 pT2 pT3		43 35 15	46.24 37.63 16.13
Regional lymph node metastasis			
N0 N1		90 3	96.77 3.23
Distant metastasis			
M0 M1		66 27	70.97 29.03
Furhman grade			
1 2 3 4		25 23 26 19	26.88 24.73 27.96 20.43

Table 2. Primer sequences used for assessing the expression of miRNAs in tumor tissue

miRNA	Stem-loop primer	Forward primer
hsa-miR-99b	5′ - GACCCACAGACACGAGCTTGTGTGCGGCGAAGGCCCGCAAGGTCGGTTCTACGGGTGGGT-GCC - 3′	5′ - TTGTGGCACCCACCCGTA-3′
hsa-miR-144	5′ - GCCCGGACTAGTACATCATCTATCTAGTGTCTCATCGCAAACTTACAGTATATGATGATATCCAGC-CAGGGCCCCA - 3′	5′ - TTGTTGGGGCCCTGGCT-3′
hsa-miR-155	5′ - ATGCTAATATGTAGGAGTCAGTTGGAGGCAAAACCCCTATCAGGATTAGCATTAAACAG-3′	5′ - GTGGGTTAATGCTAATCGTGAT-3′
hsa-miR-210	5′ - CGCTGCCAGGCACAGATCAGCCGCTGTACACGCACAGTGGGTCTGGGGCAGCGCAGTGTGCG-GTGGGCAGGGGCTGCCCTGCGCTGGAGGCACTGCCGGT-3′	5′ - GTTCTGTGCGTGTGACAG-3′
hsa-miR-222	5′ - AAGATGCCATCAGAGACCCAGTAGCCAGATGTAGCTGCTGATTACGAAAGACAGGATCTACACTGGC-TACTGAGCCATTGAGGGTACCTACACCTTCCAGCAGC-3′	5′ - GTTGCTGCTGGAAGGTGTA-3′
hsa-miR-302a	5′ - CCAAAACATGGAAGCACTTACTTCTTAGTTTCAAGCAAGTACATCCACGTTTAAAGTGGTGG-3′	5′ - GTCCACCACTTAAACGTGG-3′
hsa-miR-377	5′ - AAAGTTCCTTTGTGTGATTCAACATAAAAGCGAATTACCAAGGGCAACCTCTGCTCAA-3′	5′ - GTTTTGTGAGCAGAGGTGCC-3′

the groups was performed using the Mann–Whitney U test, qualitative — using the bilateral Pearson test. Statistically significant differences were considered at the level of $p < 0.05$.

RESULTS AND DISCUSSION

We estimated the expression of miRNA -99b, -144, -155, -210, -222, -302a, -377 in RCC patients treated with pazopanib or sunitinib with different sensitivity to targeted therapy (Table 3). In groups treated with either chemotherapeutic agent, the levels of miR-210, -222, -302a, -377 were associated with response to treatment, however, in the group receiving pazopanib, miR-99b, -144 levels in patients with a response $> 30\%$ by RECIST were higher than in patients with no tumor regression.

It was found that in tumors of patients with 0–30% response to pazopanib according to RECIST criteria, the levels of miR-99b, -222 and -377 were 2.1, 1.68 and 1.75 times lower, and the levels of miR-302a — 1.7 times higher than in patients with a response $> 30\%$ regression (Fig. 1–3).

Analysis of miR-99b, -144, -155, -210, -222, -302 and -377 expression showed that in patients with 0–30% tumor regression according to RECIST criteria after sunitinib therapy, miR-210 levels were 2.23 times higher, and the levels of miR-222, -302a, -377 were 1.95, 2.24, 1.97 times higher, respectively, than in samples, in which the regression was higher than 30%.

Based on the obtained data, we divided patients into cohorts according to the degree of response to neoadjuvant treatment: sensitive tumors (complete regression, partial regression) and resistant tumors (stabilization, progression). After analyzing the tumor tissue samples, we identified the expression parameters of the studied miRNAs, which were characteristic for 90% of sensitive RCC samples and 90% of tumors resistant to tyrosine kinase inhibitors.

As a result, we formed the expression profiles of the studied miRNAs in each cohort of patients and identified their indicators that have potential prognostic value (Fig. 3, Table 3).

There are reports on the prognostic and predictive role of several miRNAs analyzed in our study in RCC and their relationship with sensitivity to tyrosine kinase inhibitors [19–23]. Some miRNAs claimed to be oncogenic/oncosuppressive, but strict evidence about their role in renal carcinogenesis and sensitivity to targeted therapy is not established due to a contradiction and diversity of the accumulated data.

Table 3. Relationship between tumor microRNAs and RCC sensitivity to targeted therapy, $2^{-\Delta CT}$

Preparation	microRNA	Levels in 90% of sensitive tumors	Levels in 90% of resistant tumors
Pazopanib	miR-99b	> 0.6	< 0.3
	miR-144	> 0.5	< 0.3
	miR-222	> 6.1	< 3.5
	miR-302a	< 0.8	> 1.0
	miR-377	> 1.0	< 0.5
	miR-210	< 0.35	> 0.9
Sunitinib	miR-222	> 4.3	< 3
	miR-302a	> 1.3	< 0.6
	miR-377	> 0.72	< 0.4

In our study, we found that the levels of miR-99b, -144, -222, -377 did not exceed 0.3, 0.3, 3.5 and 0.5, respectively, and miR-302a values were above 1.0. In the tumor tissue of most patients with pazopanib-resistant RCC, the levels of miR-99b, -144, -222, -377 did not exceed 0.3, 0.3, 3.5 and 0.5, respectively, and miR-302a values were above 1.0. In patients with sensitive tumors, these values were more than 0.6, 0.5, 6.1, 1.0 and less than 0.8, respectively (Table 3).

High levels of miR-210 (above 0.9) and low expression of miR-222 (below 3.0), miR-302a (below 0.6) and miR-377 (below 0.4) were observed in the tissues of patients with sunitinib-resistant tumors. While in patients with sensitive tumors, high levels of miR-222, -302a, -377 (above 4.3, 1.3 and 0.72, respectively), and decreased expression of miR-210 (< 0.35) were established. There is some discrepancy between our data and the data reported in the literature wherein for miR-99b a direct correlation with sensitivity to targeted therapy, in particular pazopanib and sunitinib has been shown [20].

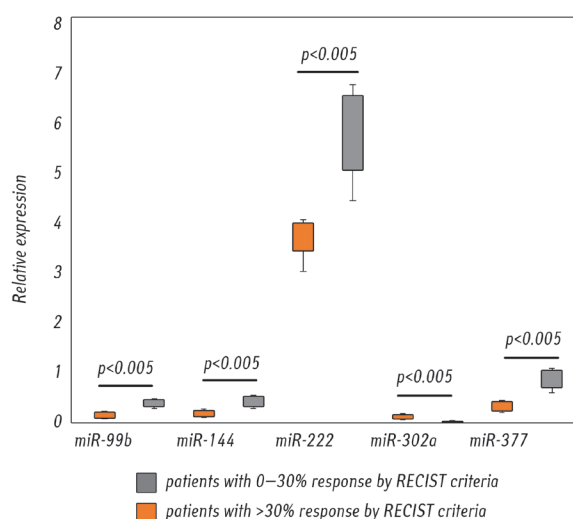


Fig. 1. miRNA expression levels in RCC tumor tissue depending on the response to pazopanib. Expression levels of miRNAs are presented as $2^{-\Delta CT}$ by boxplot with min/max whiskers

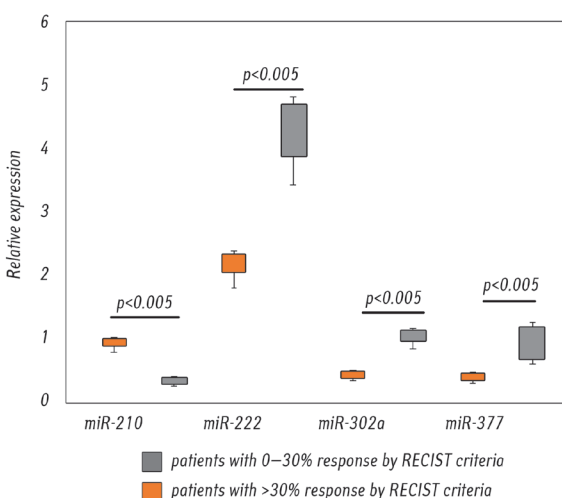


Fig. 2. miRNA expression levels in RCC tumor tissue depending on the response to sunitinib. Expression levels of miRNAs are presented as $2^{-\Delta CT}$ by boxplot with min/max whiskers

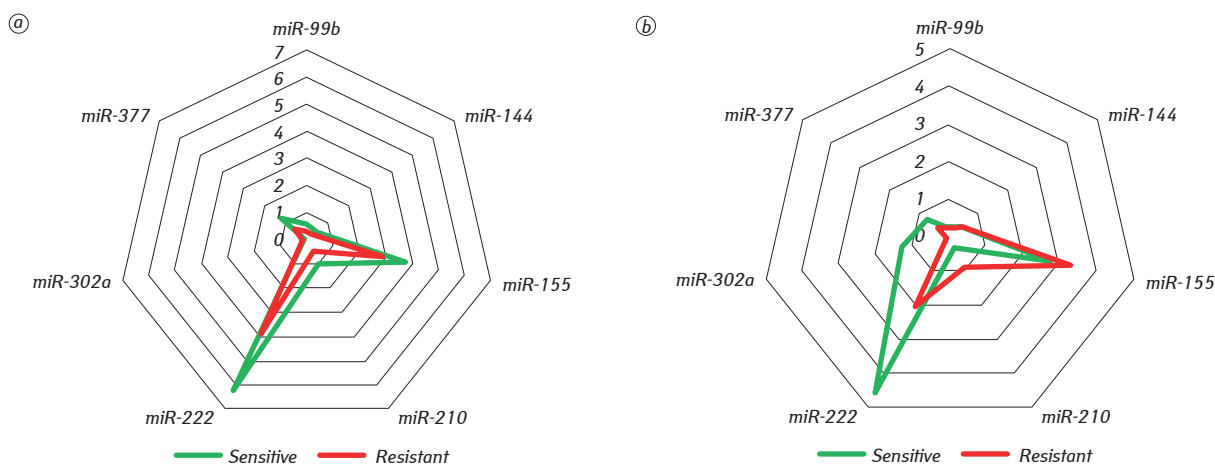


Fig. 3. Differences in miRNA expression profiles in sensitive and resistant to pazopanib (a) and sunitinib (b) RCC samples

miR-144 has not been shown to have oncogenic or oncosuppressive properties in RCC although in a number of studies miR-144 inhibited invasion and metastasis due to inhibition of MAP3K8 [24]. Nevertheless, in several studies, oncogenic properties of miR-144 were demonstrated with a direct relationship to proliferation and metastasis as well as the development of resistance to sunitinib [25].

According to Merhautova *et al.* [26], there is an association between miR-155 expression and disease progression in RCC patients receiving sunitinib. In particular, patients with higher expression of miR-155 in tumor tissue have a shorter time to disease progression after treatment with sunitinib.

miR-210, -302a and -377 are considered to be oncosuppressive in RCC cells [26–29] and miR-222 — oncogenic [30, 31]. Zhao *et al.* [32] reported elevated levels of miR-222 in RCC tissues and cell lines compared to neighboring normal tissues and the HK-2 cell line. Excessive expression of miR-222-3p promoted cell migration and invasion and inhibited apoptosis in RCC cell lines. Survival analysis showed that higher miR-222-3p expression correlated with a poor prognosis in RCC patients.

Samaan *et al.* [33] used several algorithms for prediction analysis of miR-210 targets in RCC, including the search for proteins and signaling cascades that could potentially be involved in carcinogenesis. The role of miR-210 in mitochondrial metabolism, stem cell survival, cell cycle regulation, angiogenesis, and cell adhesion was highlighted. The authors experimentally confirmed that miR-210 affects the expression of a number of hypoxia-associated genes and noted that miR-210 is an independent prognostic factor without correlation with clinical parameters such as stage or tumor size.

miRNA-302a has been shown to be linked with the sensitivity of colorectal cancer to cetuximab through the regulation of CD44, and breast cancer to cisplatin and mitoxantrone due to decreased BCRP (ABCG2) levels, indicating its significant role in carcinogenesis [34]. Rosa *et al.* [35] showed that members of the miR-302 family are involved in the differentiation

of human embryonic stem cells. Impairment of miR-302 regulation is observed in bile duct cancer and thyroid cancer. Recently, Gu *et al.* [36] demonstrated that miR-302c can inhibit the growth of hepatocellular carcinoma cells by targeting the endothelial-mesenchymal transition of endothelial cells.

Although the role of miR-377 in RCC has not been fully determined by experimental evidence, numerous bioinformatics studies and analysis of RNA sequencing in RCC clinical specimens suggest that miR-377 expression may serve as a potential biomarker for RCC progression [37].

Our results indicate the involvement of miRNAs under study in RCC response to targeted therapy. The profile of their expression might serve as the basis for the development of the predictive panel intended for the assessment of the sensitivity to the regimens of neoadjuvant RCC treatment. Such a model should include not only miRNA expression but also their regulation at the level of DNA methylation in cells exposed to target drugs, microenvironment and so-called “neighbor proteins” [38, 39]. However, one should mention some discrepancies in the profiles of miRNA expression obtained in different studies that may be explained by the differences in the characteristics of the patient cohorts under study as well as the source of the samples and the timeline of the expression analysis.

Thus, we proved the association of expression of miR-99b, -144, -222, 302a and -377 with sensitivity to pazopanib and miR-210, -222, -302a and -377 with sensitivity to sunitinib. The obtained data indicate the prospects for further study of these miRNAs in tumors of patients with kidney cancer in order to validate them as additional predictive markers of the disease.

REFERENCES

1. Stakhovskiy E, Saidakova N, Vitruk Yu. *et al.* Nephrectomy: the state of the problem in Ukraine. *Urologiya* 2017; 2: 5–13 (in Ukrainian).
2. Siegel R, Ma J, Zou Z, *et al.* Cancer statistics, 2014. *CA Cancer J Clin* 2014; 64: 9–29.
3. Cancer in Ukraine, 2019–2020. Incidence, mortality, activities of oncological service. *Bull Nat Cancer Registry Ukr*, No. 22, Kyiv, 2020. 82 p.

4. Kuusk T, Szabados B, Liu WK, *et al.* Cytoreductive nephrectomy in the current treatment algorithm. *Ther Adv Med Oncol* 2019; **11**: 1758835919879026.
5. Tanaka N, Mizuno R, Ito K, *et al.* External validation of the MSKCC and IMDC risk models in patients treated with targeted therapy as a first-line and subsequent second-line treatment: a Japanese multi-institutional study. *Eur Urol Focus* 2016; **2**: 303–9.
6. Voylenko O, Pikul MV, Stakhovskiy O, *et al.* Determination of optimal term of conduction of neoadjuvant targeted therapy in patients with localized reno–cellular carcinoma. *Klin Khir* 2019; **86**: 56–60 (in Ukrainian).
7. Stakhovskiy E, Vitruk Yu, Voylenko O, *et al.* Ways to reduce the disability of the population of Ukraine with kidney tumors. *Oncourologiya* 2017; **21**: 51–7 (in Ukrainian).
8. Tang K, Xu H. Prognostic value of meta-signature miRNAs in renal cell carcinoma: an integrated miRNA expression profiling analysis. *Sci Rep* 2015; **5**: 1–12.
9. Chekhun V, Lukianova N, Chekhun S, *et al.* Association of CD44⁺ CD24⁻/low with markers of aggressiveness and plasticity of cell lines and tumors of patients with breast cancer. *Exp Oncol* 2017; **39**: 203–11.
10. Chekhun V, Borikun T, Bazas V, *et al.* Association of circulating miR-21, -205, and -182 with response of luminal breast cancers to neoadjuvant FAC and AC treatment. *Exp Oncol* 2020; **42**: 162–6.
11. Li M, Wang Y, Song Y, *et al.* MicroRNAs in renal cell carcinoma: a systematic review of clinical implications. *Oncol Rep* 2015; **33**: 1571–8.
12. Kirkilevsky S, Krakhmalev P, Malyshok N, *et al.* Prognostic significance of microRNA-200b and ERCC1 expression in tumor cells of patients with esophageal cancer. *Exp Oncol* 2020; **42**: 167–71.
13. Voylenko OA, Stakhovsky OE, Vitruk IV, *et al.* Efficacy of neoadjuvant targeted therapy in treatment of patients with localised clear-cell renal cell carcinoma. *Adv Urol* 2021; **2021**: 6674637.
14. Kutikov A, Uzzo R. The RENAL nephrometry score: a comprehensive standardized system for quantitating renal tumor size, location and depth. *J Urol* 2009; **182**: 844–53.
15. Shparyk Ya. Kidney cancer: latest achievements in medical treatment. *Onkologiya* 2008; **10**: 436–42 (in Ukrainian).
16. Graser A, Becker C, Reiser M, *et al.* Volumetry of metastases from renal cell carcinoma: comparison with the RECIST criteria. *Der Radiologe* 2008; **48**: 850–6.
17. Ye J, Xu M, Tian X, *et al.* Research advances in the detection of miRNA. *J Pharm Anal* 2019; **9**: 217–26.
18. Wotschofsky Z, Meyer H, Jung M, *et al.* Reference genes for the relative quantification of microRNAs in renal cell carcinomas and their metastases. *Anal Biochem* 2011; **417**: 233–41.
19. Kovacova J, Juracek J, Poprach A, *et al.* Candidate microRNA biomarkers of therapeutic response to sunitinib in metastatic renal cell carcinoma: A validation study in patients with extremely good and poor response. *Anticancer Res* 2018; **38**: 2961–5.
20. Lukamowicz-Rajska M, Mittmann C, Prummer M, *et al.* MiR-99b-5p expression and response to tyrosine kinase inhibitor treatment in clear cell renal cell carcinoma patients. *Oncotarget* 2016; **7**: 78433.
21. Xiao W, Lou N, Ruan H, *et al.* Mir-144-3p promotes cell proliferation, metastasis, sunitinib resistance in clear cell renal cell carcinoma by downregulating ARID1A. *Cell Physiol Biochem* 2017; **43**: 2420–33.
22. Khella H, Butz H, Ding Q, *et al.* miR-221/222 are involved in response to sunitinib treatment in metastatic renal cell carcinoma. *Mol Ther* 2015; **23**: 1748–58.
23. D'Aniello C, Berretta M, Cavaliere C, *et al.* Biomarkers of prognosis and efficacy of anti-angiogenic therapy in metastatic clear cell renal cancer. *Front Oncol* 2019; **9**: 1400.
24. Liu F, Chen N, Xiao R, *et al.* miR-144-3p serves as a tumor suppressor for renal cell carcinoma and inhibits its invasion and metastasis by targeting MAP3K8. *Biochem Biophys Res Commun* 2016; **480**: 87–93.
25. Xiang C, Cui S, Ke Y. MiR-144 inhibits cell proliferation of renal cell carcinoma by targeting MTOR. *J Huazhong Univ Sci Technol Med Sci* 2016; **36**: 186–92.
26. Merhautova J, Hezova R, Poprach A, *et al.* miR-155 and miR-484 are associated with time to progression in metastatic renal cell carcinoma treated with sunitinib. *BioMed Res Int* 2015: 2015.
27. Yamada Y, Arai T, Kojima S, *et al.* Regulation of antitumor miR-144-5p targets oncogenes: direct regulation of syndecan-3 and its clinical significance. *Cancer Sci* 2018; **109**: 2919–36.
28. McCormick RI, Blick C, Ragoussis J, *et al.* miR-210 is a target of hypoxia-inducible factors 1 and 2 in renal cancer, regulates ISCU and correlates with good prognosis. *Br J Cancer* 2013; **108**: 1133–42.
29. Sun L, Fang Y, Wang X, *et al.* miR-302a inhibits metastasis and cetuximab resistance in colorectal cancer by targeting NFIB and CD44. *Theranostics* 2019; **9**: 8409.
30. Wang R, Ma Y, Yu D, *et al.* miR-377 functions as a tumor suppressor in human clear cell renal cell carcinoma by targeting ETS1. *Biomed Pharmacother* 2015; **70**: 64–71.
31. Lyu J, Zhu Y, Zhang Q. An increased level of MiR-222-3p is associated with TMP2 suppression, ERK activation and is associated with metastasis and a poor prognosis in renal clear cell carcinoma. *Cancer Biomark* 2020; **28**: 141–9.
32. Zhao L, Quan J, Li Z, *et al.* MicroRNA-222-3p promotes tumor cell migration and invasion and inhibits apoptosis, and is correlated with an unfavorable prognosis of patients with renal cell carcinoma. *Int J Mol Med* 2019; **43**: 525–34.
33. Samaan S, Khella HW, Girgis A, *et al.* miR-210 is a prognostic marker in clear cell renal cell carcinoma. *J Mol Diagn* 2015; **17**: 136–44.
34. Lampis A, Hahne JC, Hedayat S, *et al.* MicroRNAs as mediators of drug resistance mechanisms. *Curr Opin Pharmacol* 2020; **54**: 44–50.
35. Rosa A, Brivanlou AH. A regulatory circuitry comprised of miR-302 and the transcription factors OCT4 and NR2F2 regulates human embryonic stem cell differentiation. *EMBO J* 2011; **30**: 237–48.
36. Gu DH, Mao JH, Pan XD, *et al.* microRNA-302c-3p inhibits renal cell carcinoma cell proliferation by targeting Grb2-associated binding 2 (Gab2). *Oncotarget* 2017; **8**: 26334.
37. Yeh I, Liu KT, Shen JH, *et al.* Identification of the potential prognostic markers from the miRNA-lncRNA-mRNA interactions for metastatic renal cancer via next-generation sequencing and bioinformatics. *Diagnostics* 2020; **10**: 228.
38. Módos D, Bulusu KC, Fazekas D, *et al.* Neighbours of cancer-related proteins have key influence on pathogenesis and could increase the drug target space for anticancer therapies. *NPJ Syst Biol Appl* 2017; **3**: 1–13.
39. Lukianova NY, Borikun TV, Chekhun VF. Tumor microenvironment-derived miRNAs as prognostic markers of breast cancer. *Exp Oncol* 2019; **41**: 242–7.

ОЦІНЮВАННЯ ВІДПОВІДІ ПАЦІЄНТІВ З НИРКОВО-КЛІТИННИМ РАКОМ НА ІНГІБІТОРИ ТИРОЗИНкіНАЗ ЗА ЕКСПРЕСІЄЮ МІКРОРНК 99b, -144, -210, -222, -302a ТА -377 В ПУХЛИННІЙ ТКАНИНІ

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Стан питання: Нирково-клітинний рак є однією з найпоширеніших пухлин у дорослих. Зазвичай ці пухлини дуже стійкі до відомих методів терапії. Відомо, що профіль експресії деяких мікроРНК корелює з відповіддю хворих на нирково-клітинний рак на хіміотерапевтичні засоби.

Мета: Виявити зв'язок між експресією мікроРНК в пухлинній тканині та відповіддю на неоад'ювантну терапію

у хворих на нирково-клітинний рак. **Матеріали та методи:** За допомогою ЗТ-ПЛР аналізували рівні експресії мікроРНК-99b, -144, -155, -210, -222, -302a, -377 в пухлинній тканині 93 хворих на нирково-клітинний рак, які одержували пазопаніб або сунітиніб в неоад'ювантному режимі. Як референтну мікроРНК використовували RNU48. **Результати:** Рівні експресії мікроРНК-99b та -377 асоційовані з відповіддю на пазопаніб, а рівні експресії мікроРНК -210 та -377 — з відповіддю на сунітиніб. Характерний профіль експресії мікроРНК-99b, -144, -222, -377 та 302a визначений для 90% випадків з позитивною відповіддю на терапію пазопанібом. Аналогічно, характерний профіль експресії мікроРНК-210, -222, -302a та -377 визначено для випадків з позитивною відповіддю на терапію сунітинібом. **Висновки:** Рівні експресії мікроРНК-99b, -210 та -377 в пухлинній тканині хворих на нирково-клітинний рак можуть бути основою для розробки предикативної моделі для визначення чутливості до застосованих у неоад'ювантному режимі хіміопрепаратів у хворих на нирково-клітинний рак.

Ключові слова: мікроРНК, нирково-клітинний рак, таргетна терапія.